during therapy in patients with cGVHD, and correlating cell numbers with response.

Methods: We studied 25 adult pts with histories of hematological malignancies who developed cGVHD after allogeneic, HLA-matched HPCT. At the time of ECP initiation, pts were either dependent upon corticosteroids for control of cGvHD (21 pts), or steroid-intolerant (4 pts). A good response was defined as having > 50% reduction in the corticosteroid dose within 4 months of starting ECP, with improved or stable lesions on skin and other sites. For steroid-intolerant pts, improvement in skin condition was used to identify responders. PBMCs were analyzed before ECP began and every 2 months during ECP therapy. The numbers of plasmacytoid DCs (pDC, Lin-CD123+CD11c-HLA-DR+), myeloid DCs (mDC, Lin-CD123-CD11c+HLA-DR+), and CD4+ and CD8+ T-cells in blood were determined by flow cytometry.

Results: The median number of ECP treatments was 26 (range 2–68). Fourteen pts (56%) had good response, and 11 were non-responders. Responders had an estimated 2-yr survival of 88% after starting ECP, vs 18% for non-responders ($p = 0.004$). Responders had higher baseline numbers of pDCs (average 5.8 vs. 0.6 cells/mcL, $p = 0.025$) and mDCs (average 15 vs. 3.8 cells/mcL, $p = 0.01$) compared with non-responders. Baseline CD4+ T-cell numbers were higher in responders compared with non-responders (average 623 vs. 178 cells/mcL, $p = 0.005$), as were CD8+ T-cell numbers (712 vs. 251 cells/mcL, $p = 0.047$). There was no correlation between incidence of infection and numbers of T-cells or DCs, or response to ECP. Contrary to the original hypothesis, there were no consistent changes in the numbers of circulating DCs and T-cells among responders over a 12-month period.

Conclusion: Our results demonstrate that higher numbers of circulating DCs and T-cells predict response to ECP in pts with cGVHD. Response to ECP was significantly associated with improved survival in univariate and multivariate analyses ($p<0.03$). Our findings support a newer model for the mechanism of response to ECP therapy, involving interactions between donor-derived DCs and donor T-cells.

359

POST-TRANSPLANT IMMUNOTHERAPY FOR PEDIATRIC ALL

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Background: Pediatric Acute Lymphoblastic Leukemia (ALL) is the most common childhood malignancy. Despite substantial advancements in upfront therapy, management of patients with relapse remains one of the difficult challenges in pediatric oncology. Allogeneic transplantation can be curative for relapsing ALL, in part due to a graft versus leukemia effect. The ability to develop these approaches has been limited by lack of suitable pre-clinical models of pediatric ALL. Thus, we sought to develop a murine model of pediatric ALL to explore post-transplant immunotherapeutic strategies.

Methods: E2A-PBX1 is a translocation that occurs in approximately 5% of pediatric ALL. We utilized leukemia cells from E2A-PBX1 transgenic/CD3epsilon -/- mice that spontaneously develop precursor B cell ALL (Bijl J. et al. Gene & Development 19:224–233, 2005) to establish a transplantable ALL model.

Results: After conditioning in stem cell media, E2a-PBX1 cells could be cultured in vitro and generated leukemia consistently. The phenotype and pattern of dissemination is analogous to pediatric pre-B ALL. Immunization with dendritic cells (DC) pulsed with irradiated E2A-PBX1 cells protected against the development of leukemia. We next sought to establish a bone marrow transplant (BMT) model of minimal residual disease (MRD). Injection of E2A-PBX1 cells prior to lethal irradiation and injection of minor MHC antigen mismatched BMT resulted in the development of leukemia in two to four weeks post BMT thus mimicking a model of minimal residual disease. Ongoing experiments are exploring the ability to treat MRD with DC vaccination, adoptive T cell transfer, drugs or combination therapy.

Conclusions: We have established a transplantable model of pre-B cell ALL that mimics pediatric ALL, can be prevented by vaccines, and develop following an MRD state in allogeneic BMT. This model provides an excellent system to test strategies to diminish relapse following allogeneic transplantation for pediatric ALL.

360

DAY7 TNFR1 LEVELS FOLLOWING REDUCED INTENSITY ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT (HCT) PREDICT FOR ACUTE GVHD

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Tumor necrosis factor-α (TNF) is significant in the pathogenesis of GVHD. We have previously shown that early rises in soluble TNF receptor 1 (TNFR1) levels measured on day 7 following myeloablative (MA) allogeneic HCT predict the development of GVHD and TRM. In the setting of reduced intensity conditioning (RIC) GVHD rates are similar to MA even though GVHD onset is often delayed. We therefore hypothesized that TNFR1 levels on day 7 would correlate with GVHD after RIC. TNFR1 levels were measured on day 7 post-HCT in 156 pts (median age 56.6y, range 7–71y) who underwent RIC HCT between 2000 to 2008. Pre-HCT conditioning was Fludarabine (125–180 mg/m^2) and Busulfan (6.4–8.0 mg/kg) ± total lymphoid irradiation (2–4 Gy) in 142 pts, with minor variations in 14 pts, including 6 that received ATG. GVHD prophylaxis was tacrolimus (day -3 to 180) and short-course methotrexate (5 mg/m^2 on day 1,3,6,11 in 99 pts) or mycophenolate (day 0–28 in 57 pts). There were 47 related donor (RD) pts (30%), 109 unrelated donor (URD) pts (70%) and 34 pts received single antigen mismatched HCT (6 RD, 28 URD). The incidence of GVHD 2–4 was 49% in URD and 39% in RD. The median day of GVHD onset was 32d (range 6–162d); 56d in the RD and 30d in the URD ($p = 0.02$). The logarithm of TNFR1 levels was used for the analysis in order to normalize right-skewed values. Regression analysis revealed that a doubling in day 7 TNFR1 levels increased the risk of GVHD 2–4 by 1.36 times ($p = 0.007$), however, the risk appeared to be primarily in the URD pts [Table 1]. Consistent with this observation, the median day 7 TNFR1 level was significantly higher in pts developing GVHD 2–4 after URD (11.4 vs 11.0, $p = 0.01$), but not after RD RIC HCT (11.3 vs 11.1, $p = 0.38$). Using the median TNFR1 level in URD HCT as a threshold, we found that pts with day 7 TNFR1 levels above the median were more likely to develop GVHD 2–4 ($p = 0.008$). This correlation remained significant ($p = 0.01$) after adjustment for pt age, gender and HLA match. We conclude that for pts undergoing RIC HCT, a higher day 7 TNFR1 level correlates with incidence of GVHD 2–4. This effect of TNFR1 on GVHD was strongest in the URD setting where donor response to host alloantigens is more robust and GVHD develops more quickly. The informative value of a day 7 TNFR1 level decreases in RD pts who develop GVHD a median of 7 weeks later, suggesting that GVHD in the RD RIC setting is not strongly dependent on conditioning induced TNF release.

Association of TNFR1 with Risk of GVHD 2-4

Donor	Risk Ratio*	p-value
Overall (n=156)	1.36	0.007
RD (n=47)	1.22	0.44
URD (n=109)	1.42	0.006

*Risk Ratio is for a doubling of TNFR1 levels

361

DENILEUKIN DIFITOX (ONTAK) FOR THE TREATMENT OF ADVANCED STEROID-REFRACTORY GVHD: SINGLE INSTITUTION EXPERIENCE

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Steroid Resistant (SR)-GVHD is associated with high mortality when in encountered in allogeneic stem cell transplantation . Denileukin Diftitox (DD), a recombinant fusion protein composed of the cytocidal A chain of Diphtheria toxin and binding portion of IL-2, has potent activity against activated CD25+ T cells important in the etiology of GVHD. It has been reported that DD may have therapeutic activity for the treatment of SR-GHVD (Ho et al, BLOOD 2004). This approach was adopted within our institution for